

CONFIRMATION COPY

2/18/98

Merck & Co., Inc.

European Patent Department

DAVID A. MUTHARD

Terlings Park
Eastwick Road
Harlow
Essex CM20 2QR
England

FFR 9 1996
PATENT DEPARTMENT

Since 1st Jan. 1996 our new
fax No. is: (01279) 440717

TO: Mr. D. A. Muthard
LOCATION/COMPANY: RY 60-30
FROM: Mr. I. J. Hiscock
DATE: 07 February 1996
No. SHEETS TO FOLLOW: 25
CONF. COPY IN POST: Yes/No

MESSAGE:

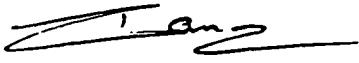
RE: WO 95/08542 KYOWA HAKKO KOGYO CO., LTD
FARNESYLTRANSFERASE INHIBITOR

Please find attached a copy of the European published patent application corresponding to the above International Application.

For your information, this application is currently with the Searching Division of the EPO and a Supplementary Search Report is expected to issue shortly. The Applicant will then be asked to confirm their intention to proceed with substantive examination of the application.

Please let me know if I can be of any further assistance.

Kind regards,


IF YOU DO NOT RECEIVE A SATISFACTORY COPY OF THIS FAX, PLEASE
TELEPHONE IAN HISCOCK ON (01279) 440175

19962
#5



Europäisches Patentamt

European Patent Office

Office européen des brevets



(11) Publication number: 0 618 221 A2

(12)

EUROPEAN PATENT APPLICATION

(21) Application number: 94302255.8

(51) Int. Cl.⁵: C07K 5/00, A61K 37/02

(22) Date of filing: 29.03.94

(30) Priority: 02.04.93 US 42377
29.06.93 US 85338

(43) Date of publication of application:
05.10.94 Bulletin 94/40

(84) Designated Contracting States:
AT BE CH DE DK ES FR GB GR IE IT LI LU MC
NL PT SE

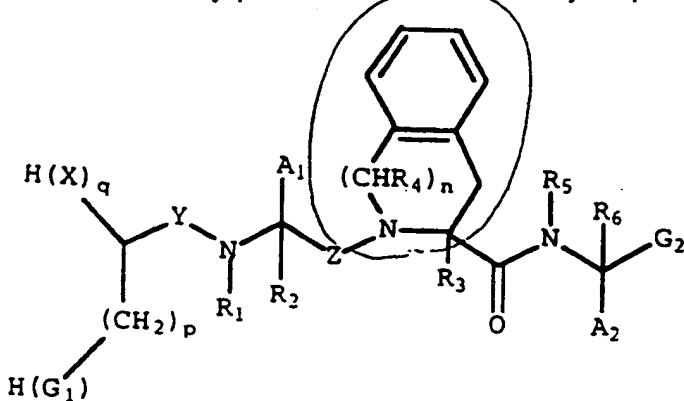
(71) Applicant: BRISTOL-MYERS SQUIBB
COMPANY
P.O. Box 4000
Princeton, NJ 08543-4000 (US)

(72) Inventor: Patel, Dinesh V.
10253 Parkwood Drive, Apt. No. 7
Cupertino, CA (US)
Inventor: Kline, Toni B.
79th Street Boat Basin
New York, NY (US)
Inventor: Meyers, Chester A.
5 Fox Trail
Medford, NJ (US)
Inventor: Leftheris, Katerina
92 Richmond Drive
Skillman, NJ (US)
Inventor: Bhide, Rajeev S.
156 Barnsbury Road
Langhorne, PA (US)

(74) Representative: Thomas, Roger Tamlyn et al
D. Young & Co.
21 New Fetter Lane
London EC4A 1DA (GB)

(54) Heterocyclic Inhibitors of farnesyl protein transferase.

(57) Inhibition of farnesyl protein transferase is effected by compounds of the formula



its enantiomers, diastereomers, pharmaceutically acceptable salts, prodrugs or solvates thereof,
wherein:

A₁ and A₂ are each independently H, alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl,
phenyl or substituted phenyl;

G₁ is S or O;

G₂ is H, -C(O)OH, -C(O)NH₂, 5-tetrazolyl, -C(O)N(R₇)OH or -CH₂OH;

X is O or R₈N;

Y and Z are each independently -CH₂- or -C(O)-;

R₁, R₂, R₃, R₄, R₅, R₆ and R₇ are each independently H or alkyl;

R₁ may also be alkanoyl;

R₁ and A₁ taken together may be -(CH₂)_m:

R₈ is H, alkyl, phenyl, phenylalkyl, substituted phenyl, (substituted phenyl)alkyl or -C(O)R₉;

R₉ is H, alkyl, phenyl, phenylalkyl, substituted phenyl or (substituted phenyl)alkyl;

m is 3 or 4;

EP 0 618 221 A2

1996
#6

(19)



Europäisches Patentamt

European Patent Office

Office européen des brevets



(11)

EP 0 856 315 A1

(12)

EUROPEAN PATENT APPLICATION

published in accordance with Art. 158(3) EPC

(43) Date of publication:

05.08.1998 Bulletin 1998/32

(51) Int. Cl.⁶: A61K 45/06, A61K 31/42

(21) Application number: 96926596.6

(86) International application number:
PCT/JP96/02241

(22) Date of filing: 08.08.1996

(87) International publication number:
WO 97/05902 (20.02.1997 Gazette 1997/09)

(84) Designated Contracting States:

AT BE CH DE DK ES FI FR GB GR IE IT LI LU MC
NL PT SE

- TANAKA, Kenji
Tsukuba-shi, Ibaraki 300-26 (JP)
- WASAWA, Yoshikazu
Tsukuba-shi, Ibaraki 300-26 (JP)

(30) Priority: 09.08.1995 JP 224659/95

(74) Representative:
Wächtershäuser, Günter, Prof. Dr.
Patentanwalt,
Tal 29
80331 München (DE)

(71) Applicant:

BANYU PHARMACEUTICAL CO., LTD.
Chuo-ku Tokyo 103 (JP)

(72) Inventors:

- YONEMOTO, Mari
Tsukuba-shi, Ibaraki 300-26 (JP)

(54) MEDICINAL COMPOSITION

(57) The present invention relates to an antitumor or anti-AIDS composition containing a protein-farnesyl-transferase inhibitor and an agent which decreases farnesyl pyrophosphate in vivo as active ingredients.

EP 0 856 315 A1

19962
#7



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : A01N 37/18, 43/40, A61K 31/445, 38/00		A1	(11) International Publication Number: WO 97/01275 (43) International Publication Date: 16 January 1997 (16.01.97)
---	--	----	---

(21) International Application Number: PCT/US96/11022 (22) International Filing Date: 26 June 1996 (26.06.96) (30) Priority Data: 60/002,251 29 June 1995 (29.06.95) US 9603091.1 14 February 1996 (14.02.96) GB	(81) Designated States: AL, AM, AU, AZ, BB, BG, BR, BY, CA, CN, CZ, EE, GE, HU, IL, IS, JP, KG, KR, KZ, LK, LR, LT, LV, MD, MG, MK, MN, MX, NO, NZ, PL, RO, RU, SG, SI, SK, TJ, TM, TR, TT, UA, US, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).
(71) Applicants (for all designated States except US): MERCK & CO., INC. [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065 (US). BANYU PHARMACEUTICAL CO., LTD. [JP/JP]; 2-3, Nihombashi, Honcho 2-chome, Chuo-ku, Tokyo 103 (JP). (72) Inventors; and (75) Inventors/Applicants (for US only): CASKEY, Charles, T. [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065 (US). NISHIMURA, Susumu [JP/JP]; 2-3, Nihombashi, Honcho 2-chome, Chuo-ku, Tokyo 103 (JP). YONEMOTO, Mari [JP/JP]; 2-3, Nihombashi, Honcho 2-chome, Chuo-ku, Tokyo 103 (JP). (74) Common Representative: MERCK & CO., INC.; 126 East Lincoln Avenue, Rahway, NJ 07065 (US).	Published With international search report.

(54) Title: COMBINATIONS OF INHIBITORS OF FARNESYL-PROTEIN TRANSFERASE

(57) Abstract

The present invention relates to compositions comprising amounts of at least two therapeutic agents selected from a group consisting of a farnesyl protein transferase inhibitor which is an effective inhibitor of the enzyme because it is competitive with respect to the protein substrate of the enzyme and a farnesyl protein transferase inhibitor which is an effective inhibitor of the enzyme because it is competitive with respect to farnesyl pyrophosphate. Further contained in this invention are methods of inhibiting farnesyl-protein transferase and treating cancer in a mammal, which methods comprise administering to said mammal, either sequentially in any order or simultaneously, amounts of at least two therapeutic agents selected from a group consisting of a farnesyl protein transferase inhibitor which is an effective inhibitor of the enzyme because it is a competitive inhibitor with respect to the protein substrate of the enzyme and a farnesyl protein transferase inhibitor which is an effective inhibitor of the enzyme because it is a competitive inhibitor with respect to farnesyl pyrophosphate, in amounts sufficient to achieve an additive or synergistic therapeutic effect. The invention also relates to methods of preparing such compositions.

1996 2
#8

Farnesyl transferase inhibitors cause enhanced mitotic sensitivity to taxol and epothilones

MARK M. MOASSER*, LAURA SEPP-LORENZINO*, NANCY E. KOHL†, ALLEN OLIFF†, AARON BALOG‡, DAI-SHI SU‡,
SAMUEL J. DANISHEFSKY‡, AND NEAL ROSEN*§¶

*Department of Medicine, †Program in Cell Biology, and ‡Program in Molecular Pharmacology and Therapeutics, Sloan-Kettering Institute, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, NY 10021; and §Department of Cancer Research, Merck Research Laboratories, West Point, PA 19486

Contributed by Samuel J. Danishefsky, December 1, 1997

ABSTRACT An important class of cellular proteins, which includes members of the p21ras family, undergoes posttranslational farnesylation, a modification required for their partition to membranes. Specific farnesyl transferase inhibitors (FTIs) have been developed that selectively inhibit the processing of these proteins. FTIs have been shown to be potent inhibitors of tumor cell growth in cell culture and in murine models and at doses that cause little toxicity to the animal. These data suggest that these drugs might be useful therapeutic agents. We now report that, when FTI is combined with some cytotoxic antineoplastic drugs, the effects on tumor cells are additive. No interference is noted. Furthermore, FTI and agents that prevent microtubule depolymerization, such as taxol or epothilones, act synergistically to inhibit cell growth. FTI causes increased sensitivity to induction of metaphase block by these agents, suggesting that a farnesylated protein may regulate the mitotic check point. The findings imply that FTI may be a useful agent for the treatment of tumors with wild-type ras that are sensitive to taxanes.

Potent and specific peptidomimetic inhibitors of farnesyl transferase (FTIs) have been synthesized and characterized by several laboratories (1–4). These compounds originally were conceived as potential anti-neoplastic drugs because the Ras family of proteins is farnesylated. Members of the ras family of protooncogenes are mutated in 30% of human cancers, and the Ras protein plays an important role in the development and progression of many human cancers. Ras is isoprenylated through the addition of a C15 farnesyl moiety. This modification confers association with the plasma membrane. Mutants of Ras that do not become membrane-associated are not transforming, and FTIs cause the reversion of transformation of fibroblasts that express the Ha-ras gene (reviewed in ref. 5).

FTIs also inhibit the growth of a majority of human tumor cells in culture. In a variety of animal systems, including v-H-ras transgenic mice and xenograft models, FTIs inhibit tumor growth, causing complete tumor regression in some murine models (6, 7). However, it is not clear that the key defarnesylated target protein is Ras. Human tumor cells without ras mutation often are quite sensitive to FTIs (8). The membrane association of Ki-ras and N-ras proteins is much less sensitive than is that of Ha-ras, yet tumor cells containing mutated Ki-ras can be quite sensitive to the drug (7, 9). Remarkably, even though FTI affects the processing of wild-type Ras protein, the drug has little discernible toxicity in animals at doses that have major anti-tumor effects (6).

These data do not rule out the possibility that Ras inhibition plays an important role in FTI action, but they suggest that

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

© 1998 by The National Academy of Sciences 0027-8424/98/951369-6\$2.00/0
PNAS is available online at <http://www.pnas.org>.

other targets may be involved (10). A number of other proteins are known to be farnesylated, including RhoB and Rap2, lamins A and B, phosphorylase kinase, rhodopsin kinase, cyclic GMP phosphodiesterase, and the γ subunit of transducin (5). Whatever the mechanism of inhibition of tumor cell growth, FTIs are novel drugs with wide therapeutic index in animals. Their role in the treatment of cancer patients has not been defined, but their low toxicity in animals, especially the absence of myelosuppression, suggests that they could be used effectively in combination with conventional chemotherapeutic agents. However, FTIs are cytostatic in some experimental models and could conceivably interfere with the effects of cytotoxic agents. We now have tested the effects of combinations of FTI and a variety of commonly used anti-cancer agents on human tumor cells in culture. FTI in combination with many of these agents causes potent and additive cell killing. Moreover, the effect of FTI in combination with taxol or an epothilone, agents that stabilize microtubule polymerization, is synergistic. Analysis of the mechanism of this interaction suggests that FTI enhances the mitotic block caused by exposure to these agents.

MATERIALS AND METHODS

Cell Culture and Growth Assays. MCF-7 and MDA-MB-468 breast cancer cells were obtained from the American Type Culture Collection and maintained in a 1:1 mixture of DME-to-F12 media supplemented with 100 units/ml penicillin, 100 µg/ml streptomycin, 4 mM glutamine, and 10% heat-inactivated fetal bovine serum and incubated at 37°C at 5% CO₂. Growth assays were performed by seeding 5,000 or 10,000 cells per well in 6-well clusters and incubating for 24 h before drug treatments. Various drug treatments then were administered as outlined for individual experiments, and cells were incubated for 8–10 days, at which time they were harvested by trypsinization and counted with a Coulter counter. Doxorubicin (Pharmacia), cisplatin (Bristol-Meyers), and taxol (Bristol-Meyers) were diluted appropriately in media to achieve the desired experimental conditions. The FTI L-744832 [Merck (6)] was dissolved in PBS, desoxyepothilone A was dissolved in dimethyl sulfoxide, and appropriate dilutions were made in media to achieve desired experimental conditions. Cells were exposed to chemotherapy for 4 h to approximate *in vivo* exposure of tumors to these drugs. FTI is used in continuous culture because preclinical studies indicate tumor regrowth upon cessation of therapy (6).

Cell Cycle Analysis. Cell cycle distribution was studied in cells harvested by trypsinization, taking care to preserve the suspended and adherent cell populations. After washing in cold PBS, cell nuclei were prepared by the method of Nusse

Abbreviations: FTI, farnesyl transferase inhibitor; FACS, flow-assisted cell sorter.

*To whom reprint requests should be addressed. e-mail: rosen@mskcc.org.

19962
#9

ACCELERATED COMMUNICATION

Paclitaxel (Taxol) Inhibits Protein Isoprenylation and Induces Apoptosis in PC-3 Human Prostate Cancer Cells

ROMANO DANESI, WILLIAM D. FIGG, EDDIE REED, and CHARLES E. MYERS

Division of Hematology/Oncology, University of Virginia, Charlottesville, Virginia 22908 (R.D., C.E.M.), and Clinical Pharmacology Branch, Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20892 (W.D.F., E.R.)

Received October 24, 1994; Accepted February 24, 1995

SUMMARY

Paclitaxel was examined for its effects on cell survival, internucleosomal DNA fragmentation, and protein isoprenylation in the human prostate cancer cell line PC-3. Treatment of cells with paclitaxel at 5–60 nM for 24 hr resulted in a dose-dependent inhibition of cell viability (IC_{50} , 31.2 nM), which was partially prevented by supplementing the cell culture medium with two nonsterol polyisoprenyl compounds, farnesyl-pyrophosphate (-PP) and geranylgeranyl-PP (3 μ M each). Furthermore, agarose gel electrophoresis of DNA extracted from cells treated with paclitaxel (15–60 nM) for 24 hr showed DNA laddering with production of fragments of 180-base pair multiples, indicating the occurrence of apoptotic cell death. Internucleosomal DNA fragmentation by paclitaxel was also detected by a photometric enzyme immunoassay using antihistone antibodies; if culture

medium was supplemented with farnesyl-PP and geranylgeranyl-PP (3 μ M each), a reduction in mono- and oligonucleosome production was observed. The post-translational incorporation of metabolites of (RS)-[5- 3 H]mevalonolactone (100 μ Ci/ml) into prenylated proteins of PC-3 cells was inhibited by paclitaxel at 30 and 60 nM. In addition, the immunoprecipitation of p21ras and p21rap-1 proteins from PC-3 cells exposed to paclitaxel (30 and 60 nM) and labeled with (RS)-[5- 3 H]mevalonolactone showed a substantial inhibition of the incorporation of farnesyl and geranylgeranyl prenoid groups, respectively, into the aforementioned proteins. These results indicate that the inhibition of protein isoprenylation is a novel component of the complex biochemical effects of the drug and plays an important role in the mechanism of paclitaxel cytotoxicity in PC-3 cells.

Eukaryotic polypeptides that are initially synthesized with the carboxyl-terminal amino acid sequence CAAX, including a variety of signal-transducing proteins such as G proteins and cGMP phosphodiesterases, can be targeted for a series of sequential post-translational modifications (1). This novel processing pathway includes the isoprenylation of the cysteine residue with a C₁₅ farnesyl or C₂₀ geranylgeranyl moiety, followed by proteolysis of the three terminal residues and α -carboxyl methyl esterification of the cysteine residue (2). The isoprenoid farnesyl-PP is a particularly important intermediate in the mevalonate pathway. It is used to synthesize cholesterol (3), and it is also bound covalently to the proteins encoded by the ras oncogenes (4), whose mutated forms are among the most common genetic abnormalities in human cancers (5). In addition, ras-related, low molecular weight G proteins, including the products of the rap-1, rab, and rho

genes, have been shown to be geranylgeranylated (1). Thus, isoprenylation is a critical step for subcellular localization of and acquisition of biological activity by signal-transducing proteins that play a pivotal role in cell growth regulation.

Inhibitors of the enzyme HMG-CoA reductase, such as lovastatin, block the production of mevalonate and its metabolites, including farnesyl-PP and geranylgeranyl-PP, and have been shown to suppress the proliferation of many cell types (6). Inhibition of isoprenoid biosynthesis by lovastatin triggers apoptosis in the human promyelocytic cell line HL-60 (7), an effect that is also produced by paclitaxel in the same cell line (8). Paclitaxel is a terpene compound obtained from the bark of *Taxus brevifolia* and is characterized by strong affinity for tubulin protein and remarkable antitumor activity *in vitro* and *in vivo* (9). Apart from its well known antimicrotubular effect, other pharmacodynamic properties of the drug are still to be examined. In the present study, the effects of paclitaxel on apoptosis and protein prenylation were investigated in the human prostate cancer cell line PC-3.

R.D. is from the Scuola Superiore di Studi Universitari e di Perfezionamento S. Anna (Pisa, Italy). Financial support from the Italian Association for Cancer Research (Milano, Italy) to R.D. is gratefully acknowledged.

ABBREVIATIONS: PP, pyrophosphate; HMG-CoA, 3-hydroxy-3-methylglutaryl-coenzyme A; bp, base pair(s); MTS, 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium; PAGE, polyacrylamide gel electrophoresis; SDS, sodium dodecyl sulfate.

**INFORMATION DISCLOSURE
STATEMENT BY APPLICANT**

(use as many sheets as necessary)

Sheet 1 of 2 Attorney Docket Number 19962YP

U.S. PATENT DOCUMENTS

FOREIGN PATENT DOCUMENTS

Examiner Signature		Date Considered	
--------------------	--	-----------------	--

*Examiner: Initial if reference considered, whether or not citation is in conformance with MPEP 609.
Draw line through citation if not in conformance and not considered.

Include copy of this form with next communication to applicant.

1. **What is your total annual income from all sources?**

**INFORMATION DISCLOSURE
STATEMENT BY APPLICANT**

(use as many sheets as necessary)

Sheet 2 of 2 Attorney Docket Number 19962YP

OTHER NON PATENT LITERATURE DOCUMENTS

Examiner Signature		Date Considered	
-------------------------------	--	----------------------------	--

*Examiner: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.